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# Effect of the $GABA_B$ agonist baclofen on dipyrone-induced delayed gastric emptying in rats

E.F. Collares and A.M. Vinagre

Departamento de Pediatria, Núcleo de Medicina e Cirurgia Experimental and Centro de Investigação em Pediatria, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brasil

## Abstract

Dipyrone administered intravenously (*iv*) or intracerebroventricularly (*icv*) delays gastric emptying (GE) in rats. Gamma-aminobutyric acid (GABA) is the most potent inhibitory neurotransmitter of the central nervous system. The objective of the present study was to determine the effect of *icv* baclofen, a $GABA_B$ receptor agonist, on delayed GE induced by dipyrone. Adult male Wistar rats received a saline test meal containing phenol red as a marker. GE was indirectly evaluated by determining the percent of gastric retention (%GR) of the meal 10 min after orogastric administration. In the first experiment, the animals were injected *iv* with vehicle ($C_{iv}$) or 80 mg/kg (240 µmol/kg) dipyrone ($Dp_{iv}$), followed by *icv* injection of 10 µl vehicle (bac0), or 0.5 (bac0.5), 1 (bac1) or 2 µg (bac2) baclofen. In the second experiment, the animals were injected *icv* with 5 µl vehicle ($C_{icv}$) or an equal volume of a solution containing 4 µmol (1333.2 µg) dipyrone ($Dp_{icv}$), followed by 5 µl vehicle (bac0) or 1 µg baclofen (bac1). GE was determined 10 min after *icv* injection. There was no significant difference between control animals from one experiment to another concerning GR values. Baclofen at the doses of 1 and 2 µg significantly reduced mean %GR induced by *iv* dipyrone ($Dp_{iv}$bac1 = 35.9% and $Dp_{iv}$bac2 = 26.9% *vs* $Dp_{iv}$bac0 = 51.8%). Similarly, baclofen significantly reduced the effect of dipyrone injected *icv* (mean %GR: $Dp_{icv}$bac1 = 30.4% *vs* $Dp_{icv}$bac0 = 54.2%). The present results suggest that dipyrone induces delayed GE through a route in the central nervous system that is blocked by the activation of $GABA_B$ receptors.

**Correspondence**
E.F. Collares
Departamento de Pediatria
Faculdade de Ciências Médicas
UNICAMP
13083-970 Campinas, SP
Brasil
Fax: +55-19-3788-7322
E-mail: pediat@fcm.unicamp.br

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- $GABA_B$ receptors
- Baclofen

## Introduction

Gastric emptying consists of the transfer of gastric content to the small bowel as a result of the action of stimulating and inhibitory mechanisms that control the motor activity of the stomach, pylorus and duodenum. Under physiological conditions, gastric emptying occurs in an appropriate manner for the conditions of digestion and absorption present in the small bowel (1,2).

Intravenous (*iv*) and intracerebroventricular (*icv*) administration of dipyrone to rats leads to a delay in gastric emptying of a liquid meal (saline). This phenomenon is more intense during the first hour after *iv* administration and is abolished by subdiaphragmatic vagotomy and electrolytic lesion of the hypotha-

lamic paraventricular nucleus (3). These observations suggest that dipyrone influences gastric emptying through the central nervous system (CNS), leading to a predominance of inhibitory mechanisms conveyed by the vagus nerve on the motor activity of the stomach, pylorus and/or duodenum. However, the neurotransmitters possibly involved in the mediation of this effect are unknown.

Gamma-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the CNS, where three different receptors have been identified: $GABA_A$, $GABA_B$ and $GABA_C$ (4-7). $GABA_A$ receptors show a predominantly postsynaptic localization and, when activated, lead to rapid membrane hyperpolarization due to an increase in $Cl^-$ channel conductance (4,5). $GABA_C$ receptors are also associated with $Cl^-$ channels, but differ from $GABA_A$ receptors in terms of pharmacological, structural, functional and genetic aspects and cellular localization (6-8). Activation of $GABA_B$ receptors through $G_i/G_o$ membrane protein signaling leads to the inhibition of adenylate cyclase, increases $K^+$ channel conductance, and reduces $Ca^{2+}$ channel conductance (5).

Studies using agonists and antagonists of GABA receptors located in neurons of the dorsal vagal complex (DVC) have provided evidence that this acid is involved in the control of gastrointestinal motility (9-13). In particular, baclofen (ß-p-chlorophenyl-GABA), a lipophilic derivative of GABA, binds as a specific agonist to $GABA_B$ receptors which, when activated, induce stimulation of the gastric acid secretion and motor activity of the stomach through the vagus nerve (11,14-16).

The objective of the present study was to test the hypothesis that activation of $GABA_B$ receptors with *icv* baclofen inhibits delayed gastric emptying induced by dipyrone in rats.

## Material and Methods

Male Wistar rats weighing 250-300 g, adapted to laboratory conditions for 2 weeks, were used. The experimental protocols applied in the present study followed the recommendations of the Brazilian College of Animal Experimentation. For surgical procedures, the animals were sedated by intraperitoneal injection of 75 mg/kg thiopental. After the procedures, the animals were kept in individual cages with ration and water available *ad libitum*.

Solutions of dipyrone or baclofen (Sigma, St. Louis, MO, USA) were prepared just before administration using sterile saline solution as vehicle, with dipyrone being protected from light. The *iv* or *icv* doses of dipyrone have been described elsewhere (3). Equivalent volumes of the vehicle were used as control.

Eight days before the study, a 15-mm 21-G stainless steel cannula was implanted into the right lateral ventricle of each animal and fixed to the skull with two screws, self-polymerizing acrylic and instant adhesive (cyanoacrylate ester). The cannulae were implanted at the following coordinates in relation to the bregma according to the atlas of Groot (17): anteroposterior = -0.2 mm, right lateral = 1.5 mm, vertical = 4.2 mm. For microinjection, a 28-G internal cannula was connected through polyethylene tubing to a 25-µl Hamilton syringe.

In the first experiment, animals were injected *iv* through a caudal vein with vehicle ($C_{iv}$) or 80 mg/kg (240 µmol/kg) dipyrone solution ($Dp_{iv}$), followed by *icv* injection of 10 µl vehicle (bac0) or an equal volume of a solution containing 0.5, 1 or 2 µg baclofen (bac0.5, 1 and 2, respectively) over a period of 30 s, with the animal remaining connected to the system for an additional 30 s. Gastric emptying was determined 10 min after removal of the internal cannula.

In the second experiment, animals received 5 µl of the vehicle ($C_{icv}$) or an equal volume of a solution containing 4 µmol (1333.2 µg) dipyrone ($Dp_{icv}$) *icv* over a period of 30 s, with the animal remaining con-

nected to the system for an additional 30 s. Using another injection system, the animals received 5 µl vehicle (bac0) or an equal volume of 1 µg baclofen solution (bac1) over a period of 30 s, with the animal remaining connected to the system for an additional 30 s. Gastric emptying was determined 10 min after removal of the internal cannula.

Gastric emptying was evaluated in the animals between 14:00 and 17:00 h after a 24-h fast during which only water was available, and which was withdrawn at the time of the test. The test meal consisted of 2 ml/100 g body weight of an aqueous 0.9% NaCl solution containing 60 µg/ml phenol red as marker. In all experiments, gastric emptying was indirectly evaluated in non-sedated animals, except for the time of sacrifice, by determining the percent of gastric retention (%GR) of the phenol red-containing test meal recovered within the gastric content 10 min after orogastric administration of the meal (18). Phenol red concentrations were measured with a spectrophotometer at 560 nm. Higher *icv* doses of baclofen (≥1.5 µg/kg) in rats have induced exploratory behavioral effects like grooming, gnawing and, in some cases, rigid posture with extended limbs and arched back (19). In the present study, the baclofen doses ranged from 2 to ~8 µg/kg and induced the reactions described above. However, these reactions did not interfere with the measurement of gastric emptying *in vivo*.

At the end of the study, all animals were sacrificed and injection into the lateral ventricle was confirmed by administering 10 µl of a 1% Evans blue solution through the cannula. The brains were then removed and fixed in 10% formalin for 24 h. Specimens were then cut into coronal sections and *icv* injection was confirmed when the dye was detected in the fourth ventricle.

The gastric retention results are reported as means ± SEM. ANOVA was used for statistical analysis and pairs were compared by the Tukey test ($\alpha = 0.05$). The dose-response effect of baclofen was determined by calculation of the correlation coefficient for ordered pairs (r).

## Results

Figure 1 shows the results of the first experiment in which *iv* administration of dipyrone caused a significant increase in the gastric retention of animals receiving vehicle *icv* (bac0) compared to controls (mean %GR: $Dp_{iv}bac0 = 51.8$ *vs* $C_{iv}bac0 = 33.2\%$). In contrast, no significant difference in gastric retention was observed between the group of animals receiving *iv* dipyrone followed by 1 and 2 µg *icv* baclofen (bac1 and bac2) and its respective control group (mean %GR: $Dp_{iv}bac1 = 35.9$ *vs* $C_{iv}bac1 = 24.2\%$ and $Dp_{iv}bac2 = 26.9$ *vs* $C_{iv}bac2 = 19.0\%$). There-

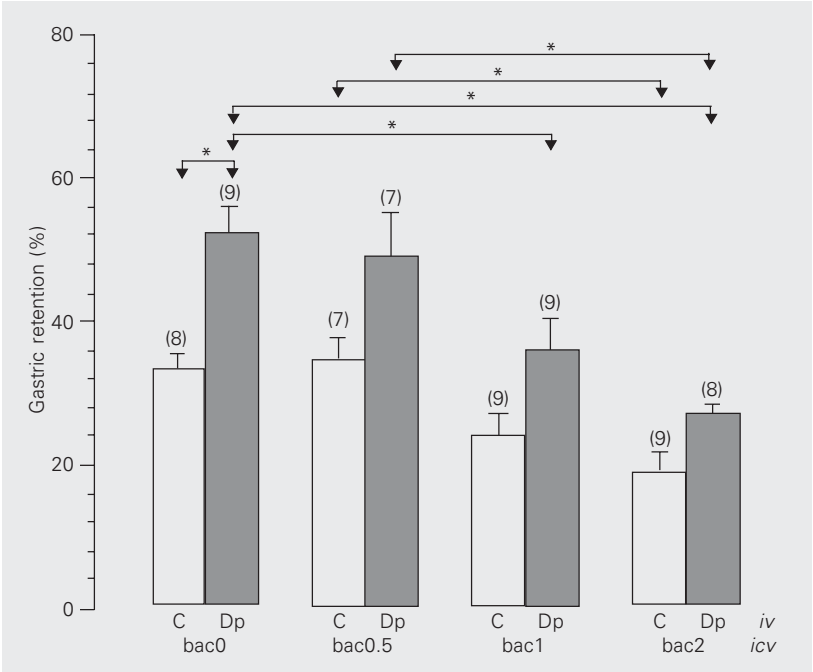


Figure 1. Gastric retention of a saline test meal 10 min after orogastric administration to rats. Eight days before the experiment, all animals were implanted with a stainless steel cannula into the right lateral ventricle. The animals were injected *iv* with vehicle (C) or 80 mg/kg (240 µmol/kg) dipyrone (Dp), followed by *icv* injection through an internal cannula of 10 µl vehicle (bac0) or an equal volume of solution containing 0.5, 1 or 2 µg baclofen (bac0.5, bac1 and bac2, respectively). Gastric retention, reported as mean ± SEM in percent, was determined 10 min after removal of the internal cannula. The number of animals in each group is given in parentheses. *P < 0.05 (Tukey test).

fore, 1 and 2 µg doses of baclofen significantly reduced gastric retention induced by *iv* dipyrone ($Dp_{iv}$bac1 and $Dp_{iv}$bac2 *vs* $Dp_{iv}$bac0). A strong negative correlation was observed between the dose of baclofen and gastric retention in animals receiving dipyrone (r = -0.97). Control animals injected *iv* with vehicle also showed a negative correlation between the dose of the $GABA_B$ agonist and gastric retention (r = -0.92), with the reduction in gastric retention being nonsignificant, except for bac0.5 *vs* bac2 controls (mean %GR: $C_{iv}$bac0.5 = 34.7 *vs* $C_{iv}$bac2 = 19.0%).

In the second experiment (Figure 2), *icv* administration of 1 µg baclofen also significantly reduced the effect of previous *icv* injection of 4 µmol (1333.2 µg) dipyrone on gastric retention of the test meal (mean %GR: $Dp_{icv}$bac1 = 30.4 *vs* $Dp_{icv}$bac0 = 54.2%). No significant difference in gastric retention was observed between the control animals injected *icv* with vehicle or baclofen (mean %GR: $C_{icv}$bac1 = 26.3 *vs* $C_{icv}$bac0 = 29.5%).

## Discussion

The mechanisms involved in the phenomenon of delayed gastric emptying induced by dipyrone in rats, or the site(s) of action of the drug in the CNS, are unknown. However, in the present study we showed that *icv* administration of baclofen, a specific $GABA_B$ receptor agonist, abolished this effect.

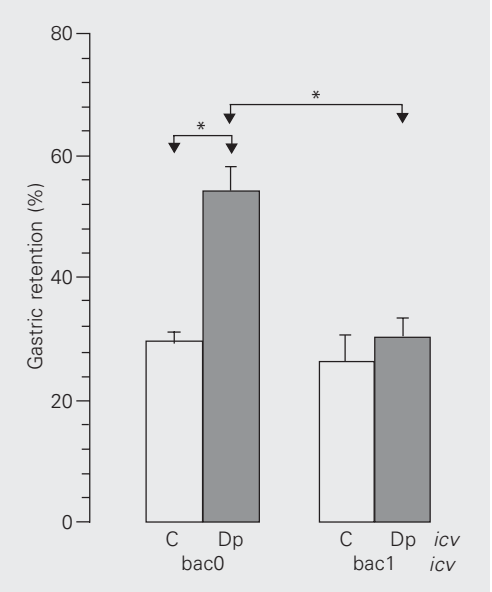


Figure 2. Gastric retention of a saline test meal 10 min after orogastric administration to rats. Eight days before the experiment, all animals were implanted with a stainless steel cannula into the right lateral ventricle. The animals were injected *icv* through an internal cannula with 5 µl vehicle (C) or an equal volume of solution containing 4 µmol (1333.2 µg) dipyrone (Dp), followed by injection of 5 µl vehicle (bac0) or an equal volume of solution containing 1 µg baclofen (bac1). Gastric retention, reported as mean ± SEM in % for N = 8 animals, was determined 10 min after removal of the internal cannula. *P < 0.01 (Tukey test).

$GABA_A$ and $GABA_B$ receptors are distributed within the rat CNS at equivalent concentrations in some areas, with a predominance of the former in most brain regions and of the latter in some others (20,21).

In the CNS, activation of $GABA_B$ receptors by baclofen results in the hyperpolarization of postsynaptic membranes or inhibition of the release of the neurotransmitter at presynaptic terminals (5). Activation of postsynaptic $GABA_B$ receptors leads to a prolonged increase in $K^+$ channel conductance, which is responsible for the generation of slow inhibitory GABA-ergic events in the CNS (4,5,22), including the modulation of rhythmic hippocampal activity (23). Presynaptic $GABA_B$ receptors are primarily involved in the regulation of neurotransmitter release, since the main effect of baclofen consists of reducing the release of excitatory and inhibitory synaptic transmitters (4,5). This action of baclofen has been observed in a variety of synapses, with the drug reducing the release of noradrenaline, dopamine, acetylcholine, serotonin, glutamate, and aspartate. Presynaptic $GABA_B$ receptors may also function as autoreceptors, reducing the release of GABA and thus diminishing the postsynaptic inhibition mediated by the activation of $GABA_A$ receptors (22).

Since in a previous study (3) the effect of dipyrone on gastric emptying could be abolished by subdiaphragmatic vagotomy, we may assume that the DVC is primarily involved in this phenomenon. The DVC is formed by the nucleus tractus solitarii whose neurons receive information through the afferent route and by the dorsal nucleus of the vagus nerve, where cholinergic stimulating and non-cholinergic, non-adrenergic inhibitory motoneurons are located, whose axons

correspond to the efferent route of the vagus nerve (2).

In the rat, $GABA_B$ receptors are located predominantly at presynaptic afferent terminals of the vagus nerve, which project into the nucleus tractus solitarii, although evidence also indicates a postsynaptic location in this nucleus (24).

One may speculate that the phenomenon of delayed gastric emptying induced by dipyrone is the result of activation of a route that stimulates inhibitory motoneurons and/or inhibits excitatory motoneurons of the vagus nerve. Since there is strong evidence that GABA plays a role in the control of gastric motility at the level of the DVC (9,11-13), activation of presynaptic $GABA_B$ receptors by baclofen that leads to the blockade of excitation of a non-cholinergic, non-adrenergic route and/or blockade of the inhibition of a cholinergic route might explain the results observed in the present study.

However, in addition to the possible involvement of the DVC which also influences gastric motility, a role of GABA in other nearby nuclei such as the nucleus ambiguus and nucleus raphe obscurus has been demonstrated (9,25). GABA receptors have also been identified in the hypothalamic paraventricular nucleus (26,27), which shows connections with the DVC and, when lesioned electrolytically, blocks the effect of dipyrone (3). In addition, the DVC is under the influence of other regions of the CNS that interfere with gastric motility, such as the central nucleus of the amygdala and the insular cortex, where GABA receptors have also been identified (20,21,28-31).

Based on the evidence showing a more extensive effect of dipyrone on the CNS, an anticonvulsant effect of this drug has been demonstrated in various experimental rat models of epilepsy. Different hypotheses to explain this effect have been proposed, such as the inhibition of prostaglandin production, blockade of the inactivation of adenosine (a potent inhibitory neuromodulator), activation of $GABA_A$ receptors, and an antiglutamatergic effect (32,33). Finally, an extrasynaptic presence of $GABA_B$ receptors has been proposed, although for these receptors to be activated continuously, elevated concentrations of GABA in the extracellular fluid are necessary or its concentration needs to be increased close to these receptors through the release from various neurons (4,23,34).

Thus, it is possible that various CNS structures and mediators are involved which, when activated, express the effect of dipyrone on gastric emptying, in addition to sites for the blockade of this effect by baclofen.

In the first experiment, although no statistically significant difference was observed between $C_{iv}$bac0 and the other baclofen controls, gastric emptying tended to be decreased following this agonist. This observation suggests that *icv* baclofen may have an effect on this gastric function *per se*. Nevertheless, this drug had a marked and statistically significant inhibitory effect on the dipyrone-induced delay of gastric emptying. The reduction of the effect of *iv* dipyrone administration followed by 1 and 2 µg *icv* baclofen was slightly higher (a 31% reduction in mean GR in the $Dp_{iv}$bac1 group and a 48% reduction in the $Dp_{iv}$bac2 group compared to the $Dp_{iv}$bac0 group) than that observed in the respective controls (a 27% reduction in mean GR in the $C_{iv}$bac1 group and a 42% reduction in the $C_{iv}$bac2 group compared to the $C_{iv}$bac0 group). Taken together, these results suggest that these two drugs have opposite effects on the CNS through independent mechanisms.

The results of the second experiment supported more convincingly the view that baclofen blocks a route that is required for the effect of dipyrone in the CNS. Baclofen induced a 4-fold reduction in the effect of dipyrone on gastric retention (a 44% reduction in mean GR for the $Dp_{icv}$bac1 group compared to the $Dp_{icv}$bac0 group) compared to that observed in the respective control (an

11% reduction in mean GR for the $C_{icv}$bac1 group compared to the $C_{icv}$bac0 group).

The present results suggest that dipyrone induces delayed gastric emptying through a route in the CNS that is blocked by the activation of $GABA_B$ receptors.

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